Hold at 4°C

[0035] For the Exonuclease VII approach 0.2µl of P^{33} αATP, 2.0µl of H-APX and H-T_{11}M are added to the preferential display product prior to the PCR reaction.

[0036] The Exonuclease III approach does not require these components, instead 2.0µl of H-APX, 2.0µl of H-T_{11}M, 0.2µl of P33αATP, 1.6µl of dNTP (25µM), 1.2µl of 10X PCR Buffer and 2.2µl of dH_2O are added. These make a 20µl total reaction volume for PCR.

[0037] Genes display is carried out using QuickPoint™ Gel system (Novex; San Diego, CA). Assay procedure is same as recommended by the manufacturer. Briefly, the precast gel is pre-electrophoresed for 5 minutes. 20 µl of PCR reaction mixed with an equal volume of QuickPoint™ sample loading buffer is then heated to 80°C for 2 minutes and 1 µl is loaded immediately onto the gel (6% polyacrylamide). 0.5 µl of the sample loading buffer is loaded onto the remaining wells to assure a straight banding pattern. Electrophoresis is carried out in QuickPoint™ cell at 1200 V for approximately 10 minutes depending upon the length of the sequences. Following electrophoresis, the glass cassette enclosing the gel is washed in water for 5 minutes on a low speed shaker, dried in an oven at 80°C for 20 minutes and exposed to X-ray film overnight to generate an autoradiogram.

[0038] The present invention has been described with reference to particular preferred embodiments; however, the scope of the invention is defined by the attached claims and should be construed to include reasonable equivalents.

Claims

What is claimed:

[c1]

1. A method to eliminate redundant sequences which are common between two or more samples using bimolecular degrading enzymatic reagents and radiolabeled assay to identify the remaining sequences which are uniquely expressed or over and under expressed in these samples, comprising of the steps:

a). isolating RNAs or DNAs from samples which are of interest,

b). generating cDNA from RNAs using RT-PCR methods and technologies,

c). cross mixing complementary cDNAs with RNAs of samples of interest,

d). degrading the hybridized complements using degrading reagents,

e). amplifying resultant cDNAs or RNAs by PCR amplification,

f). displaying and reading resultant cDNAs or RNAs, and

h). sequencing unique cDNAs or RNAs.

[c2]

2. The method of claim 1, wherein said samples of interest recited in (a) are cells, tissues, pathogens, plants, and animals which RNAs and DNAs are isolated using standard prior art methods and technologies.

[c3]

3. The samples of claim 2, are comprised of cells, tissues, pathogens, plants, and animals with little or totally known genetic sequence information.

[c4]

4. The samples of claim 2 are comprised of cells, tissues, pathogens, plants, and animals, which have differentiated due to a diseased state, developmental, change, or induced by an external or internal stimulus.

[c5]

5. The method of claim 1, wherein said RT-PCR methods and technologies is comprised of radioactive labeled to anchored oligo dT primers to generating cDNAs or RNAs.

[c6]

6. The method of claim 1, wherein said cross mixing complementary samples comprise of mixing cDNAs and RNAs from samples recited in Claims 3 and 4 forming hybridized cDNA/RNA.

[c7]

7. The method of claim 1, wherein said degrading reagents recited in d) are Exonuclease III or VII enzyme.

[c8]

8. The method of claim 1, wherein said amplifying by PCR recited in e) comprises of using AmpliTaq Gold Polymerase.

[c9]

9. The method of claim 1, wherein said displaying resultant cDNAs or RNAs recited in f) is a electrophoresis gel or capillary electrophoresis.

[c10]

10. The method of claim 1, wherein said reading resultant cDNAs or RNAs recited in f) is a photographic plate.

[c11]

11. The method of claim 1, wherein said sequencing cDNAs or RNAs recited in h) is performed by a MegaBace DNA sequencer or similar automated sequencers.

[c12]

12. A method to eliminate redundant sequences which are common between two or more samples using bimolecular degrading enzymatic reagents and fluorescence dye assay to identify the remaining sequences which are uniquely expressed or over and under expressed in these samples, comprising of the steps;

a). isolating of RNAs or DNAs from samples which are of interest,

b). generating cDNA from RNAs using RT-PCR methods and technologies,

c). cross mixing complementary cDNAs with RNAs of samples of interest,

d). degrading the hybridized complements using degrading reagents,

e). amplifying resultant cDNAs or RNAs by PCR amplification,

f). displaying and reading resultant cDNAs or RNAs, and

h). sequencing unique cDNAs or RNAs.

[c13]

13. The method of claim 12, wherein said samples of interest recited in (a) are cells, tissues, pathogens, plants, and animals which RNAs and DNAs are isolated using standard prior art methods and technologies.

[c14]

14. The samples of claim 12, are comprised of cells, tissues, pathogens, plants, and animals with little or totally unknown genetic sequence information.

[c15]

15. The samples of claim 12 are comprised of cells, tissues, pathogens, plants, and animals, which have differentiated due to a diseased state, developmental, change, or induced by an external or internal stimulus.

[c16]

16. The method of claim 12, wherein said RT-PCR methods and technologies is comprised of fluorescence dyes labeled to anchored oligo dT primers to generate cDNAs or RNAs.

[c17]

17. The fluorescence dyes of claim 16, are dyes which absorb and fluorescence at two distinct wavelengths, are fluorescence quencher pairs, fluorescence a two distinct fluorescence lifetimes, and fluorescence at two distinct polarizations.

[c18]

18. The method of claim 12, wherein said cross mixing complementary samples comprise of mixing cDNAs and RNAs from samples recited in Claims 3 and 4 forming hybridized cDNA/RNA.

[c19]

19. The method of claim 12, wherein said degrading reagents recited in d) are Exonuclease III or VII enzyme.

[c20]

20. The method of claim 12, wherein said amplifying by PCR recited in e) comprises of using AmpliTaq Gold Polymerase.

[c21]

21. The method of claim 12, wherein said displaying resultant cDNAs or RNAs recited in f) is a electrophoresis gel or capillary electrophoresis.

[c22]

22. The method of claim 12, wherein said reading resultant cDNAs or RNAs recited in f) is a scanning fluorescence spectrophotometer.

[c23]

23. The method of claim 12, wherein said sequencing cDNAs or RNAs recited in h) is by a MegaBace DNA sequencer or similar automated sequencers.

[c24]

24. A method to degrading cDNA/RNA, or cDNA/cDNA or RNA/RNA hybrids using S1 nuclease enzyme to eliminate common cDNA's or RNA's from two different cell and tissue types.

[c25]